Aflatoxicol: structure of a new transformation product of aflatoxin B_1

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The incubation of aflatoxin B_1 with a steroid-hydroxylating fungus yielded a cyclopentane ring reduction product with reduced biological activity.

Our preliminary report (1) described the isolation and biological activity of a fungal transformation product from aflatoxin B_1. Tentatively the name "aflatoxicol" or aflatoxin R_0 was assigned to the compound. On the basis of further investigations of this substance, we propose that aflatoxicol is a reduction product of aflatoxin B_1 (**1**), as represented by Formula **2**.

Since the parent aflatoxin B_1 molecule is a carcinogen, we have investigated the biological activity of this new transformation compound. Under conditions specified for the duckling biliary hyperplasia assay (2), 56.0 μg B_1 had the same biological activity as 1.0 mg of aflatoxicol (1), making this compound 18 times less toxic than aflatoxin B_1 by histopathological assay.

Dactylium dendroides NRRL 2575 was cultivated for 60 h in Fernbach flasks containing 500 ml of yeast extract – sucrose medium and 60 mg of aflatoxin B_1. The mycelia were extracted with $CHCl_3/CH_3OH$ (20:1), and the extracts were

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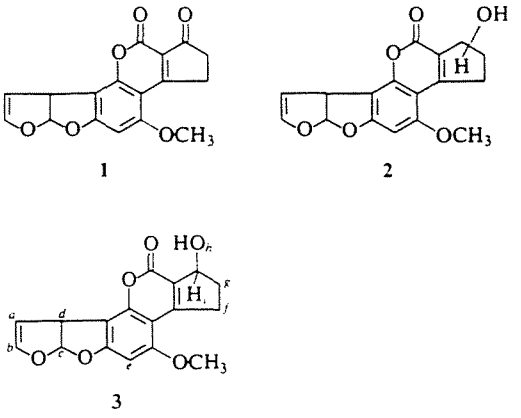

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chromatographed on preparative thin-layer chromatographic (t.l.c.) plates. The fluorescent (aflatoxicol) bands were scraped from the preparative t.l.c. plates and the aflatoxicol was eluted from the silica gel with methanol through a Millipore filter system. The transformation product (aflatoxicol) was further purified by silicic acid and silica gel G-HR[2] (Brinkmann) column chromatography. The fractions containing aflatoxicol were pooled, dried, and dissolved in $CHCl_3$. Aflatoxicol was precipitated by adding 8 volumes of *n*-hexane to the chloroform extract. On a silica gel G-HR t.l.c. plate developed with acetone/chloroform (10:40), aflatoxicol had an R_f of 0.57 as compared with an R_f of 0.69 for aflatoxin B_1.

The ultraviolet spectrum of the parent molecule aflatoxin B_1, $C_{17}H_{12}O_6$, molecular weight 312, exhibited λ_{max}^{MeOH} at 362, 265, 254 mμ (ε 21 800, 13 400, 7000, respectively), and infrared bands at $\nu_{max}^{CHCl_3}$ 1760 (intense), 1685 (weak), 1632, 1590, 1600, 1130, and 2850 cm^{-1}.

The ultraviolet spectrum of aflatoxicol exhibited λ_{max}^{MeOH} at 325, 261, and 254 mμ (ε 14 100, 10 800, 6790), which was dissimilar to that of the parent aflatoxin B_1 molecule. This dissimilarity is noted in the hypsochromic shift from 362 mμ in B_1 to a 325 mμ major absorption peak for aflatoxicol. This observation was compared to the properties of a reduction product of aflatoxin B_1, tetrahydrodesoxoaflatoxin, which lacks a carbonyl function on the cyclopentane ring (3). This compound had a similar absorption spectrum pattern, λ_{max}^{MeOH} 332, 265, and 255 mμ (ε 13 900, 9200, 8500), to the aflatoxicol molecule. This initial ultraviolet comparison of tetrahydrodesoxoaflatoxin and aflatoxicol to aflatoxin B_1 indicated a functional change or disappearance of a carbonyl function from B_1 yielding aflatoxicol. As already mentioned briefly, the infrared spectrum of aflatoxin B_1 possesses a high intensity absorption at 1760 cm^{-1} and a low intensity band at 1685 cm^{-1}. These two absorption bands in the aflatoxin B_1 spectrum are attributable to the unique structural relationship of the coumarin and ketone carbonyl groups in the molecule as shown by Asao *et al.* (3). The infrared spectrum (KRS-5 plate, multiple internal reflectance) of aflatoxicol was similar to that of aflatoxin B_1 (bands at 1590 cm^{-1} (phenyl), 1600 cm^{-1} (—HC=CH—), 1130 cm^{-1} (C—O—C), 2850 cm^{-1} (—O—CH_3), and other strong bands at 1485, 1440, and 1360 cm^{-1}), but lacked the two absorption bands for the coumarin and ketone carbonyl functions observed for B_1 at 1760 and 1685 cm^{-1}. The absence of these two absorption bands in aflatoxicol showed that the unique structural relationship of two carbonyls was no longer prevalent in aflatoxicol. In addition, aflatoxin B_1 contains no infrared bands above 3300 cm^{-1} excluding the presence of hydroxyls; however, a broad absorption band at 3400 cm^{-1} for aflatoxicol indicated the presence of a hydroxyl function. The vibrations involving the stretching of the C—O bond give rise to a strong infrared band at 1070 cm^{-1} indicating the presence of a secondary alcohol. The presence of a strong band at 1720 cm^{-1} indicated the presence of the carbonyl band of the unsaturated δ-lactone ring in the aflatoxicol moiety. This spectral evidence, observed for aflatoxicol and compared to spectral data compiled for tetrahydrodesoxoaflatoxin and related coumarin-nucleus derivatives by Asao and co-workers (3, 4), appeared to be presumptive evidence that aflatoxicol contained a reduced carbonyl in the cyclopentane ring.

The molecular formula of aflatoxicol was established by high-resolution mass spectrometry; the largest mass peak was observed at $m/e = 314$, and the mass calculated for $C_{17}H_{14}O_6$ was 314.00, indicating a dihydro derivative of aflatoxin B_1. In the fragmentation pattern, a m/e 296 peak

[2]The mention of firm names or trade products does not imply that they are recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

does arise from the loss of a molecule of H_2O from the aflatoxicol molecule but this peak is absent in the B_1 spectrum. Furthermore, it is important to note that the parent B_1 molecule, m.w. 312, does not yield a 294 peak since a molecule of H_2O cannot be split out of the carbonyl function upon fragmentation.

A comparison of the n.m.r. spectra of aflatoxin B_1 and aflatoxicol reveals their striking similarity, especially in the difuran and cyclopentane moieties. The following peaks were observed for aflatoxicol (**3**): olefinic proton H_a, triplet, δ 5.49; olefinic proton H_b, triplet, δ 6.5; H_c, doublet, δ 6.84; H_d, multiplet, δ 4.80; H_e, singlet, δ 6.37, assigned to the aromatic proton via a comparative spectral study with sterigmatocystin (3); H_f, multiplet, δ 3.27; and the three-proton singlet at δ 4.02 for the methoxy group (O—CH_3). The observed splitting patterns and chemical shifts of the protons were identical to those of the parent molecule, aflatoxin B_1 (3), and indicated no changes in the difuran ring. A multiplet at δ 2.55 was observed for aflatoxin B_1 while the aflatoxicol spectrum differed in having a signal at δ 2.25 (multiplet, H_g). The signals given by **3** at δ 2.42 (multiplet, H_h) and δ 5.31 (multiplet, H_i) can be assigned, respectively, to a hydroxyl proton (^2H_2O exchange) and to a methine proton linked to the same carbon atom as the hydroxyl moiety.

The above evidence demonstrates that *Dactylium dendroides* NRRL 2575 is capable of reducing the carbonyl group on the cyclopentane ring of aflatoxin B_1.

Acknowledgments

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